Problem Set #4

DD 2020

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**Introduction**

A novel cell surface receptor was recently identified in a patient-derived pancreatic cancer cell line and was named EHR1*.* Following up on the findings, two more EHR cell surface proteins of the same family were identified (EHR2 and EHR3). The three members of this family all have extracellular receptor domains, each with a transmembrane region and cytosolic kinase. It appears that there are at least four common ligands to all three members of the family. Following ligand binding to the extracellular domain of the EHR receptor, heterodimerization is initiated which activates downstream activity. It appears that once activated, there is a significant increase in cell proliferation, differentiation and cell survival, making the pancreatic tumor cells very resistant to conventional therapy.

The project is at an early stage and your first task is to determine if there is a suitable cell-surface target in the EHR family to develop an antibody to treat metastatic pancreatic cancer. It is hypothesized that by binding to any or all of the EHR members with a monoclonal antibody, heterodimerization would be prevented, making the cancer much less aggressive and more vulnerable to treatment. The scientific team proposes that developing an antibody-drug conjugate may provide the increased efficacy needed to treat this aggressive cancer.

**Problem 4.1 Selection of Surface EHR target**

The scientific/medical collaboration enrolled eight pancreatic cancer patients with advanced disease into a study to determine the level of expression of EHR-1, EHR-2 and EHR-3 protein in pancreatic tumor tissue. The patients in the EHR study agreed to an endoscopic biopsy to obtain samples of both the cancer tissue and nearby healthy tissue.

A mouse monoclonal antibody for each EHR protein was available for use in quantitating EHR density on cell surfaces using Surface Plasmon Resonance.

The murine mouse antibodies are not suitable candidates as a therapeutic antibodies. The pancreatic tissue samples from both healthy and cancer tissue were analyzed and their EHR protein expression level results are summarized in Table 4.1.

Table 4.1. EHR cell surface protein expression levels (Molecules/m2) on cell surfaces, for each of the eight patients sampled.

EHR-1 EHR-2 EHR-3

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Patient | Healthy | Cancer | Healthy | Cancer | Healthy | Cancer |
| 1001 | 76 | 1872 | 655 | 1489 | 89 | 654 |
| 1002 | 103 | 2085 | 136 | 855 | 94 | 56 |
| 1003 | 36 | 1620 | 89 | 1540 | 54 | 410 |
| 1004 | 85 | 1540 | 1488 | 1650 | 78 | 580 |
| 1005 | 77 | 1963 | 1672 | 1430 | 65 | 68 |
| 1006 | 102 | 1452 | 89 | 102 | 15 | 54 |
| 1007 | 45 | 3125 | 705 | 1945 | 56 | 62 |
| 1008 | 72 | 842 | 968 | 780 | 90 | 48 |

**Overall, which cell surface proteins would you select to be the target of the antibody development program and why?**

**4.2 Selection of Human IgG1 Antibody**

The team has decided to go ahead with the development an antibody for the treatment of pancreatic cancer by targeting the selected EHR cell surface protein, named the pancreatic cancer cell marker (PCCM). Your challenge is to select the most appropriate human antibody derived from phage display technology. There were three human antibodies that were engineered (hAb116, hAb201 and hAb302) and are available for evaluation. Each of the antibodies was evaluated against each patients cancer cell line, using plasmon resonance techniques. The antibody association rate constant (ka) and dissociation rate constants (kd) are summarized in Table 4.2, for each tissue sample taken.

***-Determine the antibody dissociation constants (KD) for each patient.***

***-Determine the mean values for each antibody KD with 95% confidence limits.***

***-Select the best binding antibody for development and defend your decision***

Table 4.2. Association and dissociation rate constants for the three antibodies binding to the PCCM.

hAb116 hAb201 hAB302

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Patient | ka  (M-1 s-1) | kd  (s-1) | ka  (M-1 s-1) | kd  (s-1) | ka  (M-1 s-1) | kd  (s-1) |
| 1001 | 3.1 105 | 1.5 10-4 | 1.5 105 | 1.9 10-4 | 3.5 105 | 9.8 10-4 |
| 1002 | 4.6 105 | 1.6 10-4 | 4.1 105 | 2.5 10-4 | 4.1 105 | 7.3 10-4 |
| 1003 | 5.6 105 | 1.3 10-4 | 3.2 105 | 3.6 10-4 | 2.8 105 | 12.8 10-4 |
| 1004 | 4.2 105 | 1.8 10-4 | 1.8 105 | 4.9 10-4 | 5.7 105 | 9.7 10-4 |
| 1005 | 8.9 105 | 3.0 10-4 | 1.9 105 | 8.7 10-4 | 4.1 105 | 13.0 10-4 |
| 1006 | 3.4 105 | 1.2 10-4 | 2.4 105 | 4.7 10-4 | 3.2 105 | 14.8 10-4 |
| 1007 | 4.5 105 | 1.1 10-4 | 1.5 105 | 4.8 10-4 | 5.5 105 | 6.9 10-4 |
| 1008 | 7.2 105 | 1.8 10-4 | 3.2 105 | 7.6 10-4 | 8.2 105 | 13.2 10-4 |

**4.3 Optimization of FcRn**

The selected antibody is taken forward into preclinical development, named Pancomab (marketing made their contribution here). You decide to explore the effect of optimizing the FcRn domain making modifications to the Fc receptor and inventing three new antibodies (PancomabF, G and H). The purpose of the optimization is to try to extend the pharmacokinetic half-life. As a final selection criterion for the human antibody, cyno monkey pharmacokinetic evaluations were done at a 1 mg/kg IV bolus dose. The monkeys had an average weight of 5 kg and a plasma volume estimated to be 40 mL/kg.

- **Determine the volume of distribution and elimination half life of each of the Pancomab antibodies in Table 4.3 and summarize your results.**

-**Compare the pharmacokinetic parameters of the four antibodies and select one of them for clinical development.**

**Table 4.3 Mean Pharmacokinetic results (ug/mL) in cyno monkeys (n=6) for the four different human antibodies.**

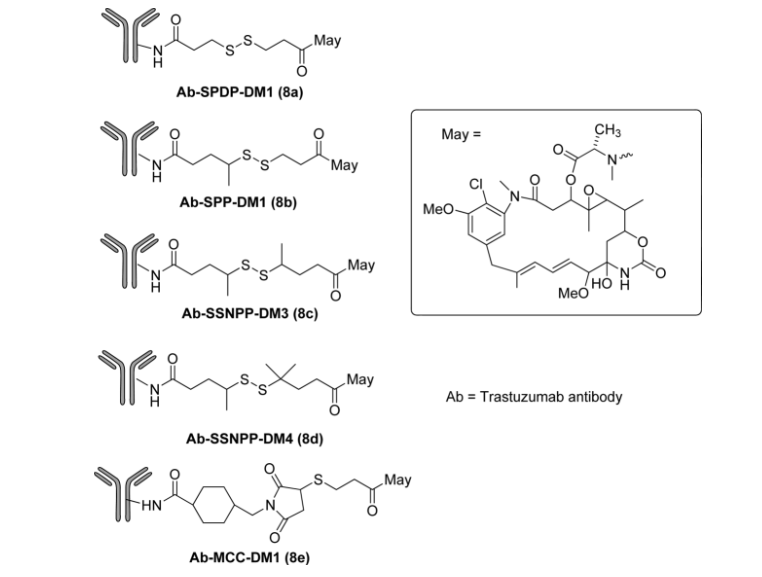
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Time in days** | **Pancomab** | **PancomabF** | **PancomabG** | **PancomabH** |
| 0.5 | 21 | 23 | 24 | 24 |
| 1 | 17 | 21 | 23 | 24 |
| 1.5 | 15 | 20 | 22 | 23 |
| 2 | 12 | 18 | 21 | 22 |
| 2.5 | 11 | 17 | 20 | 22 |
| 3 | 9 | 16 | 19 | 21 |
| 3.5 | 7 | 14 | 18 | 21 |
| 4 | 6 | 13 | 18 | 20 |
| 5 | 4 | 11 | 16 | 19 |
| 6 | 3 | 10 | 15 | 18 |
| 7 | 2 | 8 | 14 | 17 |
| 8 | 1 | 7 | 13 | 16 |
| 10 | 1 | 5 | 11 | 15 |
| 12 | 0.4 | 4 | 9 | 13 |
| 14 | 0.2 | 3 | 7 | 12 |
| 16 | 0.1 | 2 | 6 | 11 |

**4.4 Optimization of Antibody Toxin Linker**

The toxin chosen for the ADC project is DM1, the question is what is the linker?

Five different linkers were developed by your chemistry group; 4 different disulfide bond analogs and one thioether analog were synthesized, with structures shown in Figure 1. ADCs were prepared using the different linkers, each containing an average of three DM1s per Pancomab human antibody. The structures of the five ADCs are shown in Figure 1. The antibody/toxin is now named Pancomab 8. Mice xenograph studies were run with a relevant human tumor cell line (taken and expanded from one of the patient tissues samples) and tumor volumes were followed in the mice.

Figure 1. Structural representation of Pancomab-maytansinoid ADCs.



Pancomab8a

Pancomab8b

Pancomab8c

Pancomab8d

Pancomab8e

1. **In a review by A. Talkington and R. Durrett (Bull Math Biol., 2015, *in class reading materials*), the exponential model for tumor growth is presented. It the simplest of tumor models and is also the most often used. The model equation is shown below in Equation 1. Fit the tumor growth data in Table 4 to the exponential model for tumor growth rate and compare the rates of growth for the different ADCs.**

**V = V0 e(r t) Eq. 1**

V = volume of mouse tumor as a function of time in mm3

V0 = initial volume of tumor in mm3

r = rate of tumor growth (inverse days or inverse hours)

t = time (days or hours)

1. **Select the antibody toxin linker based on a comparison of the growth kinetics and give a brief rationale for your choice**
2. **How strong is the evidence that DM1 conjugation improves efficacy in this animal model**

**Table 4.4. Comparison of the *in vivo* efficacy of pancomab ADCs as a function of linker. A single IV dose of 10mg/kg in a xenograph mouse model, tumor model from a human pancreatic cancer line expressing EHR-1, grade 3 by IHC. The in life portion of the study was run for three weeks; all ADCs were well tolerated by the mice at the 10 mg/kg single dose used. The results shown are estimates of tumor volume in mm3, average of 3 animals in each group. The uncertainty in tumor volume measurement is +/- 20%.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Time in days** | **Pancomab**  **No DM1** | **Pancomab8a** | **Pancomab8b** | **Pancomab8c** | **Pancomab8d** | **Pancomab8e** |
| 0 | 190 | 170 | 160 | 180 | 170 | 190 |
| 3 | 240 | 230 | 220 | 210 | 200 | 190 |
| 6 | 330 | 290 | 270 | 270 | 240 | 210 |
| 9 | 440 | 370 | 350 | 310 | 280 | 240 |
| 12 | 600 | 480 | 430 | 370 | 320 | 280 |
| 15 | 820 | 600 | 500 | 450 | 380 | 300 |
| 18 | na | 770 | 640 | 520 | 440 | 330 |
| 21 | na | na | 780 | 640 | 500 | 360 |

**4.5 Repeat Dose IV Toxicity in Cynomolgus Monkeys,**

Cynomolgus monkeys were selected as the primate toxicology species. Acute (single dose) and repeat dose studies were be done. However, the most interesting data in the most sensitive species come from the repeat dose (4 total doses given every 2 weeks) studies in monkeys, in which there were observed significant changes in ALT levels.

The mean ALT levels (U/L) for the monkeys studied (n=16 per dose group), after repeat dosing, are listed in Table 4.5 for Pancomab8. The doses tested were

0 mg/kg Pancomab8, 2, 6, 12 and 24 mg/kg. The DM1 to antibody ratio (DAR) was 3.7:1 on a mole basis, determined analytically.

Table 4.5. Elevations in liver transaminase (ALT in U/L) as a function of dose and duration for Cynomolgus Monkeys.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Week | Dose  0 mg/kg | Dose  2 mg/kg | Dose  6 mg/kg | Dose  12 mg/kg | Dose  24 mg/kg |
| 0 | 41 | 46 | 44 | 45 | 39 |
| 1 | 42 | 48 | 44 | 48 | 39 |
| 2 Dose | 40 | 51 | 49 | 65 | 141 |
| 3 | 39 | 41 | 48 | 58 | 134 |
| 4 Dose | 45 | 39 | 42 | 68 | 168 |
| 5 | 43 | 38 | 56 | 56 | 120 |
| 6 Dose | 42 | 44 | 41 | 75 | 178 |
| 7 | 45 | 42 | 52 | 52 | 109 |
| 8 Dose | 41 | 38 | 54 | 89 | 169 |
| 9 | 39 | 38 | 56 | 54 | 115 |
| 10 | 37 | 45 | 41 | 42 | 96 |
| 11 | 45 | 49 | 40 | 41 | 89 |
| 12 | 42 | 38 | 42 | 44 | 82 |

***-***

***Discussion of Maximum Tolerated Dose***



-**Estimate the maximum tolerated dose for Pancomab8 in the cynomolgus monkey species. Consider the variability in the data and the recovery following the last dose. Explain your reasoning.**

**-The guidance for starting dose from the FDA and Biologics is somewhat complex, open to interpretation. For simplicity, we will assume that the starting dose is ½ of maximum tolerated dose established in the monkey studies. This proposed starting dose can be countered by the FDA reviewers. Suggest a starting dose for the human phase 1 clinical trial of Pancomab8.**

**4.6 Troubles in the Phase 1 Study**

The manufacturing campaign to support the Phase 1 studies had some difficulties. The first problem was low yield, low enough to make it impossible to use one lot (Lot 801) to support the entire Phase 1 study. A second lot (Lot 802) was manufactured, but failed due to an aseptic protocol violation. At that time, management decided to go ahead with a third manufacturing run (very expensive) and to start the Phase 1 studies with the first lot, planning on resupply of the study with the third lot (Lot 803), once released.

The early results at low doses of Pancomab8 showed real promise. The first two dose levels (using Lot 801 only) showed very little toxicity and two of the patients demonstrated a clinical response at the second dose level. At that point, the third lot (LOT 803) was introduced into the study. Moderate to severe liver toxicity was observed at the second dose level in three patients dosed with LOT 803. The study was stopped and the program is now under review.

A preliminary investigation of the three lots produced for the Phase 1 study included the antibody binding data (plasmon resonance), the DM1 content and human pharmacokinetic results. The summary biopharmaceutical data are shown in Table 4.6.

Table 4.6. Analytical characterization and pharmacokinetic properties of Pancomab8 Lots used in the Phase 1 study.

Antibody Binding DM1 characterization PK/PD (n=3 each lot)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Lot number | ka  (M-1 s-1) | kd  (s-1) | DAR | % DM1 as free drug | Average t1/2  Pancomab8 | Peak ALT (U/L) |
| 801 | 5.6 105 | 1.3 10-4 | 3.4 | <0.1% | 16 days | 52 |
| 802 | 6.2 105 | 0.9 10-4 | 3.2 | <0.1% | na | Na |
| 803 | 3.8 105 | 3.0 10-4 | 6.8 | 0.9% | 7 days | 1100 |

-**Propose an explanation of why the third lot showed toxicity and poor PK performance**

**-Propose a remedy to the problem for the release of future lots**

**-Should the Phase 1 study be repeated with better lots of Pancomab8, and what should the antibody properties look like?**